

DIVERSITY AND MORPHOLOGY OF THE SPECIES OF *PSEUDO-NITZSCHIA* (BACILLARIOPHYTA) OF THE NATIONAL PARK SISTEMA ARRECIFAL VERACRUZANO, SW GULF OF MEXICO

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ABSTRACT

In order to evaluate the risk for marine ecosystem and human health, the diversity and morphology of diatoms of the genus *Pseudo-nitzschia* were studied. Weekly sampling was performed at seven geo-referenced sites in the National Park Sistema Arrecifal Veracruzano, SW Gulf of Mexico, during the annual cycle from May 2005 to May 2006. Samples were taken in the 30-cm surface layer of the water column with a phytoplankton net of 20- μ m mesh size. With the use of light, transmission and scanning electron microscopy, seven *Pseudo-nitzschia* species were identified: non-toxic *P. cf. subcurvata* (examined only by light microscopy) and *P. brasiliana*, and potentially toxic, causative agents of amnesic shellfish poisoning (ASP) due to domoic acid: *P. calliantha*, *P. cuspidata*, *P. delicatissima*, *P. pseudodelicatissima* and *P. pungens*. It is concluded that there is a potential risk of ASP in the study area. *P. brasiliana* and *P. calliantha* are new records for the SW Gulf of Mexico. Comparisons with studies conducted in the northern Gulf of Mexico indicate that the diversity of *Pseudo-nitzschia* in the Gulf of Mexico may be greater than currently documented and that some species (e.g., *P. multiseriata*) may have biogeographic limitations (e.g., temperatures below 27 °C).

Key words: amnesic shellfish poisoning, diatoms, Gulf of Mexico, morphology, new records, *Pseudo-nitzschia*, taxonomy, Veracruz.

RESUMEN

Con el fin de evaluar el riesgo para el ecosistema marino y la salud humana, se estudió la diversidad y la morfología de las diatomeas del género *Pseudo-nitzschia* del Parque Nacional Sistema Arrecifal Veracruzano, en el suroeste del Golfo de México. Se realizaron muestreos semanalmente en siete sitios georeferenciados durante un ciclo anual de mayo de 2005 a mayo de 2006. Las muestras se tomaron de la capa superficial de 30 cm de la columna de agua con una red fitoplanctónica de 20 μm de tamaño de malla. Con ayuda de los microscopios fotónico, electrónico de transmisión y electrónico de barrido se identificaron siete especies de *Pseudo-nitzschia*: las no tóxicas *P. cf. subcurvata* (estudiada solamente en el microscopio fotónico) y *P. brasiliiana*, y las potencialmente venenosas, causantes de la intoxicación amnésica por consumo de mariscos (ASP) debido al ácido domoico: *P. calliantha*, *P. cuspidata*, *P. delicatissima*, *P. pseudodelicatissima* y *P. pungens*. Se concluye que existe riesgo potencial de ASP en el área de estudio. *P. brasiliiana* y *P. calliantha* son nuevos registros para el suroeste del Golfo de México. Las comparaciones con los estudios en el norte del mismo cuerpo marino indican que la diversidad de *Pseudo-nitzschia* en el Golfo de México puede ser más alta que la documentada en el presente, y que algunas especies (e.g., *P. multiseriis*) pueden tener limitaciones ecológicas y biogeográficas (e.g., temperaturas menores de 27 °C).

Palabras clave: diatomeas, Golfo de México, intoxicación amnésica por consumo de mariscos, morfología, nuevos registros, *Pseudo-nitzschia*, taxonomía, Veracruz.

INTRODUCTION

Pseudo-nitzschia H. Peragallo is a widely distributed marine genus (Hasle & Syvertsen, 1996; Hasle, 2002), with 38 currently described species (Guiry, 2011). Interest in the genus increased when, in 1987, severe intoxications and at least three human mortalities were reported in those who consumed blue mussels (*Mytilus edulis* L.) containing the neurotoxin domoic acid (DA) (Wright et al., 1989). Further research determined that the DA was produced by the planktonic diatom *Pseudo-nitzschia multiseriis* (Bates et al., 1989). The resultant shellfish poisoning was termed amnesic shellfish poisoning (ASP), as one of the distinguishing symptoms of acute exposure to DA is short-term memory loss (Perl et al., 1990). Later, marine fish, bird and mammal mortalities occurred, caused by their consumption of DA-contaminated *Pseudo-nitzschia* species or vectors, causing DA poisoning (DAP);

thus, toxigenic *Pseudo-nitzschia* species also became a threat to marine animal health (Trainer et al., 2008).

At present, the following *Pseudo-nitzschia* species are known as DA producers: *P. pseudodelicatissima* (Hasle) Hasle, *P. pungens* (Grunow ex Cleve) Hasle, *P. multiseriis* (Hasle) Hasle, *P. seriata* (Cleve) H. Peragallo, *P. australis* Frenguelli, *P. calliantha* Lundholm, Moestrup et Hasle, *P. cuspidata* (Hasle) Hasle, *P. fraudulentata* (Cleve) Hasle, *P. multistriata* (Takano) Takano, *P. delicatissima* (Cleve) Heiden, *P. turgidula* (Hustedt) Hasle and *P. galaxiae* Lundholm et Moestrup (Bates & Trainer, 2006; Trainer et al., 2008, 2009).

Economic losses caused by DA-associated *Pseudo-nitzschia* blooms have been reported from various countries around the world: Canada, USA, Mexico, Costa Rica, Argentina, Denmark, Scotland, Ireland, France, Spain, Italy, Tunisia, Morocco, Korea and Vietnam (Skov et al., 1997; Akallal & Mouradi, 2000; Bates & Richard, 2000; Kotaki et al., 2000; Sarno & Dahlmann, 2000; Cho et al., 2002; Fehling et al., 2004; Vargas-Montero & Freer, 2004; Lundholm et al., 2005; Badylak et al., 2006; Nezan et al., 2006; Almandoz et al., 2007; Bogan et al., 2007; Cortés-Altamirano & Sierra-Beltrán, 2008; Quijano-Scheggia et al., 2009; Sarahraoui et al., 2009).

At least six of the 12 *Pseudo-nitzschia* species known to produce DA (*P. australis*, *P. delicatissima*, *P. pseudodelicatissima*, *P. multiseriis*, *P. pungens* and *P. seriata*) have caused blooms or intoxications in the Mexican Pacific and the coastal waters of the Gulf of California (Ochoa et al., 1996; Meave del Castillo & Hernández-Becerril, 1998; Licea et al., 2000; Moreno-Ruiz et al., 2004; Sierra-Beltrán et al., 2005; Cortés-Altamirano & Sierra-Beltrán, 2008). *Pseudo-nitzschia* species have also been observed in both the southern (Meave del Castillo et al., 2000; Gómez-Aguirre et al., 2004; Licea et al., 2004) and northern Gulf of Mexico (Fryxell et al., 1990, 1991; Dortch et al., 1997; Parsons et al., 1998, 2002; Liefer et al., 2009; Del Rio et al., 2010; MacIntyre et al., 2011). The following *Pseudo-nitzschia* species have been documented in the northern Gulf of Mexico: *P. brasiliana* Lundholm, Hasle et G. A. Fryxell, *P. decipiens* Lundholm et Moestrup, *P. delicatissima*, *P. prolongatoides* (Hasle) Hasle, *P. pseudodelicatissima*, *P. pungens*, *P. multiseriis*, *P. subfraudulenta* (Hasle) Hasle, *P. subpacificata* (Hasle) Hasle, and *P. galaxiae* Lundholm et Moestrup (Conger et al., 1972; Licea, 1992; Parsons et al., 1998; Lundholm & Moestrup, 2002; Lundholm et al., 2002; Krayevsky et al., 2009). *Pseudo-nitzschia* species regularly bloom in Louisiana coastal waters (Dortch et al., 1997; Del Rio et al., 2010), likely stimulated by intensifying eutrophication over the past 50 years in response to higher nutrient loads from the Mississippi River (Parsons et al., 2002). The concern that these *Pseudo-nitzschia* blooms could result in a DA event was manifested when DA was detected in gulf menhaden (Clupeidae: *Brevoortia*

patronus Goode; Del Rio et al., 2010) and in bottlenose dolphins (*Tursiops truncatus* (Montagu); Schwacke et al., 2010), demonstrating that DA is moving up into higher trophic levels in the Gulf of Mexico.

As DA produced by *Pseudo-nitzschia* has affected higher trophic levels in the northern Gulf of Mexico, further assessment of the potential threat of DA to ecosystem health was deemed necessary for the SW Gulf of Mexico. The main purpose of this study, therefore, was to document the diversity and morphology of *Pseudo-nitzschia* species of the National Park Sistema Arrecifal Veracruzano (NPSAV), Gulf of Mexico, to evaluate the risk for the marine ecosystem and human health.

MATERIAL AND METHODS

Samples were taken from May 2005 to May 2006, at seven oceanographic stations during a weekly monitoring of red tides of the Aquarium of Veracruz (Fig. 1; for geographic coordinates, see Okolodkov, 2008), in the 30-cm surface layer, using a phytoplankton net of 20- μ m mesh and 28 cm in diameter. Preliminary identifications of *Pseudo-nitzschia* species were made using a Nikon Eclipse T100 inverted microscope. Diatom frustules were cleaned by removing the organic matter by oxidation (Lundholm et al., 2002). Two milliliters of saturated KMnO_4 solution with 30% H_2SO_4 were added to a 10-ml aliquot of the sample. After 24 h, the sample was bleached by adding 10 ml of saturated oxalic acid solution, and then it was repeatedly washed with distilled water.

Thirteen samples from the seven stations taken in 2005 were selected, on the basis of their high abundance of *Pseudo-nitzschia* species, for further analysis using differential interference contrast (DIC) microscopy and electron microscopy (EM) to identify the various *Pseudo-nitzschia* species present: May 17 (st. 3, 5 and 7), May 24 (st. 3 and 5), May 31 (st. 2 and 3), June 7 (st. 1 and 6), July 13 (st. 3), August 24 (st. 3), and September 13 (st. 2). High total abundances of *Pseudo-nitzschia* species were found in May-July 2005 (up to 4.6×10^5 cells/l), August (up to 1.6×10^6 cells/l), September (up to 8.8×10^5 cells/l) and October (up to 7.1×10^5 cells/l) (Y.B. Okolodkov, unpubl. data). Starting from late October, the abundance drastically diminished, usually varying between 1.0×10^3 and 5.0×10^3 cells/l, and only once showed a high number of cells (8.9×10^4 cells/l at st. 1, on November 15, the highest abundance observed during the period from early October through mid-February).

DIC microscopy was used to determine the general groups of *Pseudo-nitzschia* present (e.g., *P. multiseriata/pungens*, *P. delicatissima* complex, *P. brasiliensis/linea*,

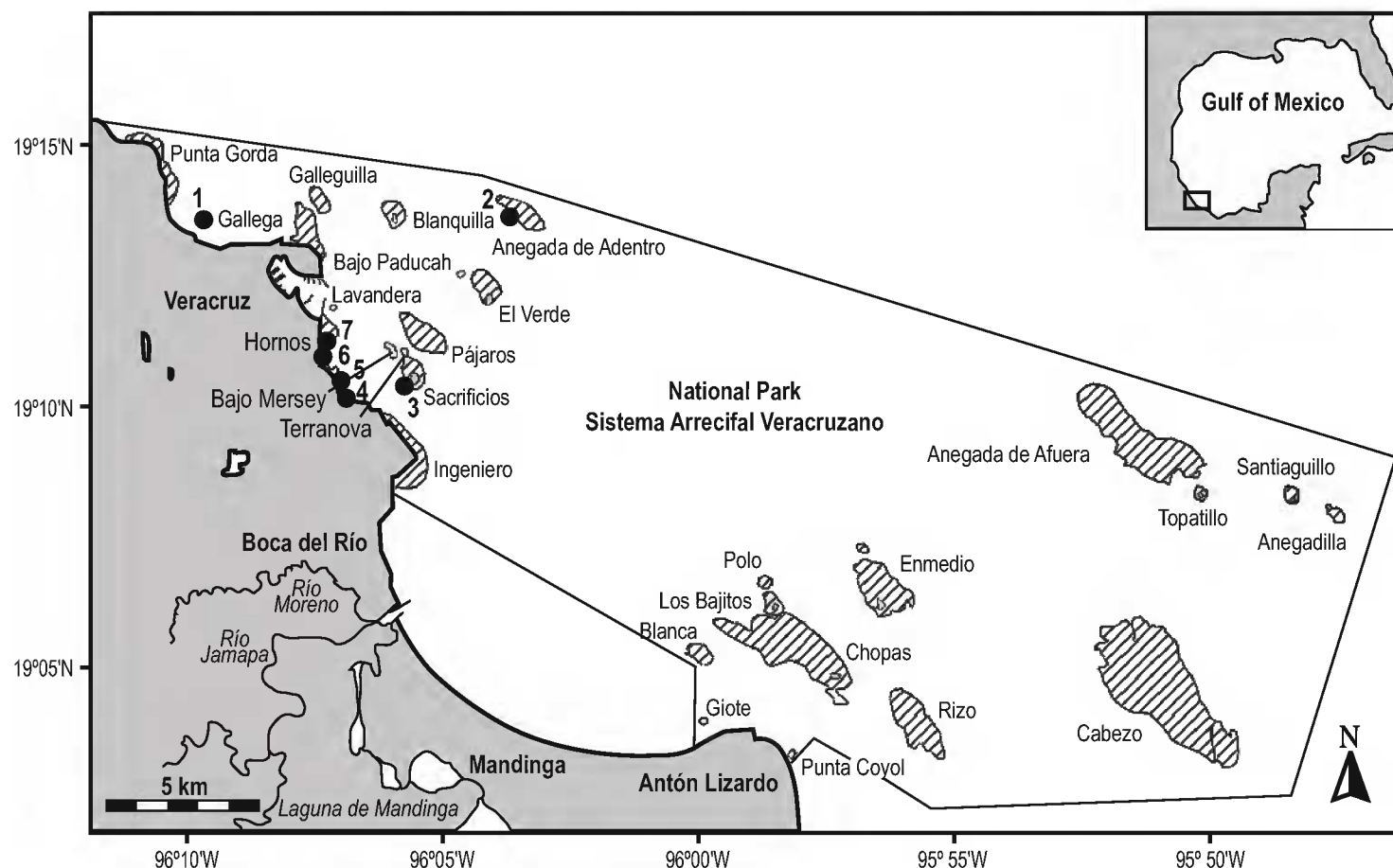


Fig. 1. Sampling sites (filled circles) in the National Park Sistema Arrecifal Veracruzano, Gulf of Mexico. Hatched areas are coral reefs.

and *P. subfraudulenta/fraudulenta*), which also facilitated EM analysis by providing some level of diversity to be expected. Valve lengths were also recorded during DIC examination. Cell length measurements made via DIC microscopy could not always be coupled with EM observations due to the inability to identify many species (i.e., the above groups) during light microscopy observation. For this reason, the data on the variability of the cell length for four species out of seven are absent. While scanning electron microscopy (SEM) was adequate for identifying most of the *Pseudo-nitzschia* species encountered, transmission electron microscopy (TEM) was needed to distinguish among the various species within the “*pseudodelicatissima/cuspidata* complex” where examination of the poroid hymen structure (Lundholm et al., 2003) was required.

Frustule samples for microscope analysis were processed according to Parsons et al. (1999). Samples were boiled in concentrated HNO_3 for 20 min, and then washed six times with distilled water. The frustules were then resuspended in 1 ml of deionized, distilled water and transferred to a coverslip (DIC), stub (SEM) or grid (TEM) for examination. For DIC microscopic analysis, 0.2 ml of suspension was transferred to a 25 mm, No. 1 square coverslip (Fisher Scientific, 12-548C, Pitts-

burgh, PA, USA), dried on a hot plate (Fisher Scientific Isotemp, Pittsburgh, PA, USA) at low setting, and permanently mounted in Naphrax (Northern Biological Supplies, Ipswich, UK). These prepared slides were then examined on an Olympus BX51 microscope fitted with DIC optics at 600x and 1000x magnification.

For SEM, 0.1 ml of the suspension was transferred to a 15 mm, round coverslip (Ted Pella, Inc., Prod. No. 26024, Redding, CA, USA), which was then affixed to a pin-style, 0.5" aluminum mount (Ted Pella, Inc., Prod. No. 16084-1, Redding, CA, USA) with PELCO 12 mm conductive, adhesive pads (Ted Pella, Inc., Prod. No. 16111, Redding, CA, USA). Stubs were air dried overnight and then sputter-coated using a Fullam EMS-76 prior to examination on a Hitachi 3400SN SEM at a working distance of 10 mm and a voltage of 25 kV. For TEM, ~0.025 ml of the suspension was transferred to a copper grid, size 300, and covered with carbon (Electron Microscopy Sciences, Hatfield, PA, USA) for observation in a JEOL JEM1400 TEM. All EM work was conducted at the Florida Fish and Wildlife Research Institute, St. Petersburg, Florida, USA.

The following morphometric measurements were made to assist in taxonomic determination (Table 1): cell length (DIC); valve width (SEM and TEM); number of fibulae and striae per 10 μm , number of rows of poroids in each stria (SEM and TEM); poroid number per 1 μm (TEM); and the pattern of perforations in the hymen of each poroid (TEM). Additionally, the valve shape and the shape of its extremities (DIC and SEM) were considered.

RESULTS

Description of the genus

***Pseudo-nitzschia* H. Peragallo in H. et M. Peragallo, 1900**

Type (lectotype): *Pseudo-nitzschia seriata* (Cleve) H. Peragallo in H. et M. Peragallo, 1900.

The following description is based on the diagnosis by Hasle (1993).

Species of *Pseudo-nitzschia* form chain colonies characterized by overlapped cells. Each cell contains two chloroplasts, each located closer to the extremities, and a central nucleus. Individual cells and colonies can move, gliding in the longitudinal direction. The cingulum consists of a series of linear bands. For identification, the cells should be examined in valve view. The frustules are usually linear or lanceolate

in both valve and girdle view, but some species are asymmetric along the apical axis in valve view. The number of interstriae is equal or approximately double compared to the number of fibulae, and one or more rows of poroids between the striae are present. The raphe is eccentric and not elevated above the valvar surface, and the raphe wall lacks poroids. Two raphes of two adjacent cells are located one against other. Some species have a central nodule (= central interspace). Using the light microscope, it can be observed as a larger space between the central fibulae.

Hasle (1965) and Hasle & Syvertsen (1996) divided *Pseudo-nitzschia* into two groups: the “*seriata* complex” (valve width >3 μm) and the “*delicatissima* complex” (<3 μm). Lundholm et al. (2002, 2003) further clarified two more groups (the “*americana* complex” and the “*pseudodelicatissima/cuspidata* complex”); the former is distinguished via light microscopy by its valves with rounded ends and shorter lengths (<65 μm). The “*pseudodelicatissima/cuspidata* complex” is distinguished morphologically primarily by differences in the structure of the poroid hymens and girdle bands (Lundholm et al., 2003).

Table 1. Morphometric characteristics of *Pseudo-nitzschia* species found in the National Park Sistema Arrecifal Veracruzano.

Species	Length (μm)	Width (μm)	Striae in 10 μm	Fibulae in 10 μm	Poroids in 1 μm
<i>P. brasiliana</i>	23-40	2.0-2.7	24-25	22-24	9.0-9.5
“ <i>delicatissima</i> ” complex ¹	40-85				
<i>P. calliantha</i>		1.4-1.6	33-40	15-22	4.1-6.5 ^H
<i>P. cuspidata</i>		1.5-1.7	36-41	19-23	6.0-6.8 ^{HH}
<i>P. delicatissima</i>		1.3-1.4	33-36	21-24	9.1-11.4
<i>P. pseudodelicatissima</i>		1.1-1.6	35-44	17 ^L -23	5.0-7.0
<i>P. pungens</i>	93-126	2.8-3.2	11-14	11-15	3.0-3.4
<i>P. cf. subcurvata</i>	28-36	1.9-2.5			

¹ Measurements of the cell length for the four species of the “*delicatissima* complex” listed below are given for all of them considering impossible to distinguish between them in light microscope.

^H Upper end of measurements is higher than those in literature (Lundholm et al., 2003).

^{HH} Upper end of measurements is higher than those in literature (Hasle, 1965; Skov et al., 1999; Lundholm et al., 2003).

^L Lower end of measurements is lower than those in literature (Lundholm et al., 2003, 2006).

Description of species

1. *Pseudo-nitzschia brasiliiana* Lundholm, Hasle et G. A. Fryxell, 2002 (Pl. 1, Fig. 1; Pl. 2, Fig. 1)

Cells are narrow, linear, with widely rounded extremities, 23-40 μm long, 2.0-2.7 μm wide, overlapped 1/8-1/11 of the cell length. 22-24 striae in 10 μm and 24-25 fibulae in 10 μm (Table 1). Two rows of poroids more or less circular, 9.0-9.5 in 1 μm , tending to form the third row near the valve margins. Central interspace is absent.

Taxonomic note. This species belongs to the “*americana* complex”. Unlike the “*seriata* complex”, the species of the “*americana* complex” have rounded valvar poles, slightly shorter cells, and weaker silicification. Unlike the “*delicatissima* complex”, the valves are wider and more robust. The number of fibulae and striae in the specimens from NPSAV is within that reported by other authors (19-28 fibulae and striae in 10 μm in Lundholm et al., 2002, and Quijano-Scheggia et al., 2008, 2011).

A new record for the SW Gulf of Mexico.

Toxicity. The species has not yet been reported to be toxic (Lundholm et al., 2002).

2. *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle, 2003 (Pl. 1, Fig. 2)

Cells are very narrow, linear, 40-85 μm long, 1.4-1.6 μm wide. The degree of overlap between cells in colonies is unknown (it is not given in the original description by Lundholm et al., 2003). 33-40 striae in 10 μm and 15-22 fibulae in 10 μm . One row of relatively large circular poroids, 4.1-6.5 in 1 μm . The hymen is discontinuously perforated, consisting of 4-9 peripheral sectors and a central area; the poroids having the lower number of sectors are always closer to the valve opposite to that with smaller fibulae and poroids. Central interspace is present.

Taxonomic note. This species belongs to the “*delicatissima* complex” (Hasle & Syvertsen, 1996) and the “*pseudodelicatissima/cuspidata* complex” (Lundholm et al., 2003). The numbers of fibulae and striae in the specimens from NPSAV are within those reported by others (19-20 and 39-40 in 10 μm , respectively, in Bargu et al., 2004; 15-22 and 34-39 in 10 μm in Lundholm et al., 2003; 15-26 and 30-40 in 10 μm in Quijano-Scheggia et al., 2008).

A new record for the SW Gulf of Mexico. It was identified for the first time in the Gulf of Mexico in Florida (Lundholm et al., 2003). Recently, it was reported in Louisiana waters, where it was dominant among other *Pseudo-nitzschia* species (Del Rio et al., 2010).

Toxicity. Some strains of this species are toxic (Lundholm et al., 1997, 2003; Bates & Trainer, 2006). However, Orlova et al. (2008) did not detect any toxicity in a strain of *P. calliantha* from the Sea of Japan, and strains from the southern Gulf of St. Lawrence, Canada, were also below the limit of detection for DA (S. Bates, pers. comm.).

3. *Pseudo-nitzschia cuspidata* (Hasle) Hasle, 1974 emend. Lundholm, Moestrup et Hasle, 2003 (Pl. 1, Fig. 3)

Bas.: *Nitzschia cuspidata* Hasle, 1965.

Cells are very narrow, linear or lanceolate, with acute extremities, 1.5-1.7 μm wide, overlapped 1/5-1/6 of the cell length. 36-41 striae in 10 μm and 19-23 fibulae in 10 μm . One row of relatively large poroids, 6.0-6.8 in 1 μm . The hymen is usually perforated by four sectors (each partially divided), so that the perforation is not continuous. Central interspace is present.

Taxonomic note. This species belongs to the “*delicatissima* complex” (Hasle & Syvertsen, 1996) and the “*pseudodelicatissima/cuspidata* complex” (Pl. 2, Fig. 2-4; Lundholm et al., 2003). The number of fibulae and striae in the specimens from NPSAV are within those reported by others, however, the poroids are somewhat denser (14-22 and 29-39 in 10 μm and 4-6 poroids in 1 μm , respectively, in Hasle, 1965, and Skov et al., 1999; 19-25 and 35-44 in 10 μm and 4-6 poroids in 1 μm in Lundholm et al., 2003). The hymen in *P. cuspidata* and *P. pseudodelicatissima* is very similar, so that it is not always possible to distinguish between these two species even in TEM; principally, they are distinguished by the shape of the valves (tapering from the middle part of the valve towards the extremities in *P. cuspidata* and tapering only near the extremities in *P. pseudodelicatissima*) and by the cell width (relatively thinner in *P. pseudodelicatissima*) (Lundholm et al., 2003).

In the SW Gulf of Mexico, the species has been previously reported from Tuxpan, Veracruz, México (Lundholm et al., 2003).

Toxicity. Reports prior to 2003 about the toxicity of *P. cuspidata* are not reliable due to confusion of this species with *P. pseudodelicatissima* (Lundholm et al., 2003). However, more recent studies have confirmed the toxicity of *P. cuspidata* (Trainer et al., 2009).

4. *Pseudo-nitzschia delicatissima* (Cleve) Heiden, 1928 (Pl. 1, Fig. 4)

Bas.: *Nitzschia delicatissima* Cleve, 1897.

Syn.: *Nitzschia actydropbila* Hasle, 1965.

Cells are very narrow, linear or lanceolate, 40-85 μm long, 1.3-1.4 μm wide, overlapped 1/7-1/10 of the cell length. 33-36 striae in 10 μm and 21-24 fibulae in 10 μm . Two rows of small poroids. The hymen has a continuously perforated area, 9.1-11.4 poroids in 1 μm . Central interspace is present.

Taxonomic note. This species belongs to the “*delicatissima* complex” (Hasle & Syvertsen, 1996). The number of fibulae in the specimens from NPSAV is within that reported by Hasle (1965), i.e., 19-25 in 10 μm .

Toxicity. This species has never caused any natural toxic event (Skov et al., 1999). However, the presence of the DA was registered in cultures (Smith et al., 1991; Rhodes et al., 1998). DA is reported as undetectable in most culture studies on *P. delicatissima* (Villac et al., 1993; Lundholm et al., 1994; Fehling et al., 2005).

5. *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle, 1993 emend. Lundholm, Moestrup et Hasle, 2003 (Pl. 1, Fig. 5)

Bas.: *Nitzschia pseudodelicatissima* Hasle, 1976.

Syn.: *Nitzschia delicatula* Hasle, 1965.

Cells are very narrow, linear, 40-85 μm long, 1.1-1.6 μm wide, symmetric in relation to the apical axis, with more or less acute extremities, overlapped 1/5-1/6 of the cell length. 35-44 striae in 10 μm and 17-23 fibulae in 10 μm . One row of small, circular, ovoid or more or less quadrate poroids with rounded angles, 5.0-7.0 in 1 μm . The hymen has 1 to 4 perforated areas (partially divided), so that the perforation is not continuous. Central interspace is present.

Taxonomic note. This species belongs to the “*delicatissima* complex”. The number of poroids in the specimens from NPSAV is slightly higher compared to those reported by others (4-6 in 1 μm in Hasle, 1965; 5-6 in Takano & Kuroki, 1977, and Rivera, 1985; 4.8-5.5 in Lundholm et al., 2003). Also, the number of striae and fibulae are higher (29-33 and 16-19 in 10 μm , respectively, in Licea, 1992).

Toxicity. Certain strains of this species are toxic (Pan et al., 2001 [now known to be *P. pseudodelicatissima* instead of *P. sp. cf. pseudodelicatissima*, M. Parsons pers. obs.]; Bates & Trainer, 2006; Moschandreu et al., 2010), although more often non-toxic strains have been reported (see references in Moschandreu et al., 2010).

6. *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle, 1993 (Pl. 1, Fig. 6)

Bas.: *Nitzschia pungens* Grunow ex Cleve, 1897.

Cells linear or lanceolate, symmetrical in relation to the apical axis, 93-126 μm long, 2.8-3.2 μm wide, overlapped 1/3-1/4 of the cell length. 11-14 striae in 10 μm and 11-15 fibulae in 10 μm . Two rows of large, circular poroids, 3.0-3.4 in 1 μm .

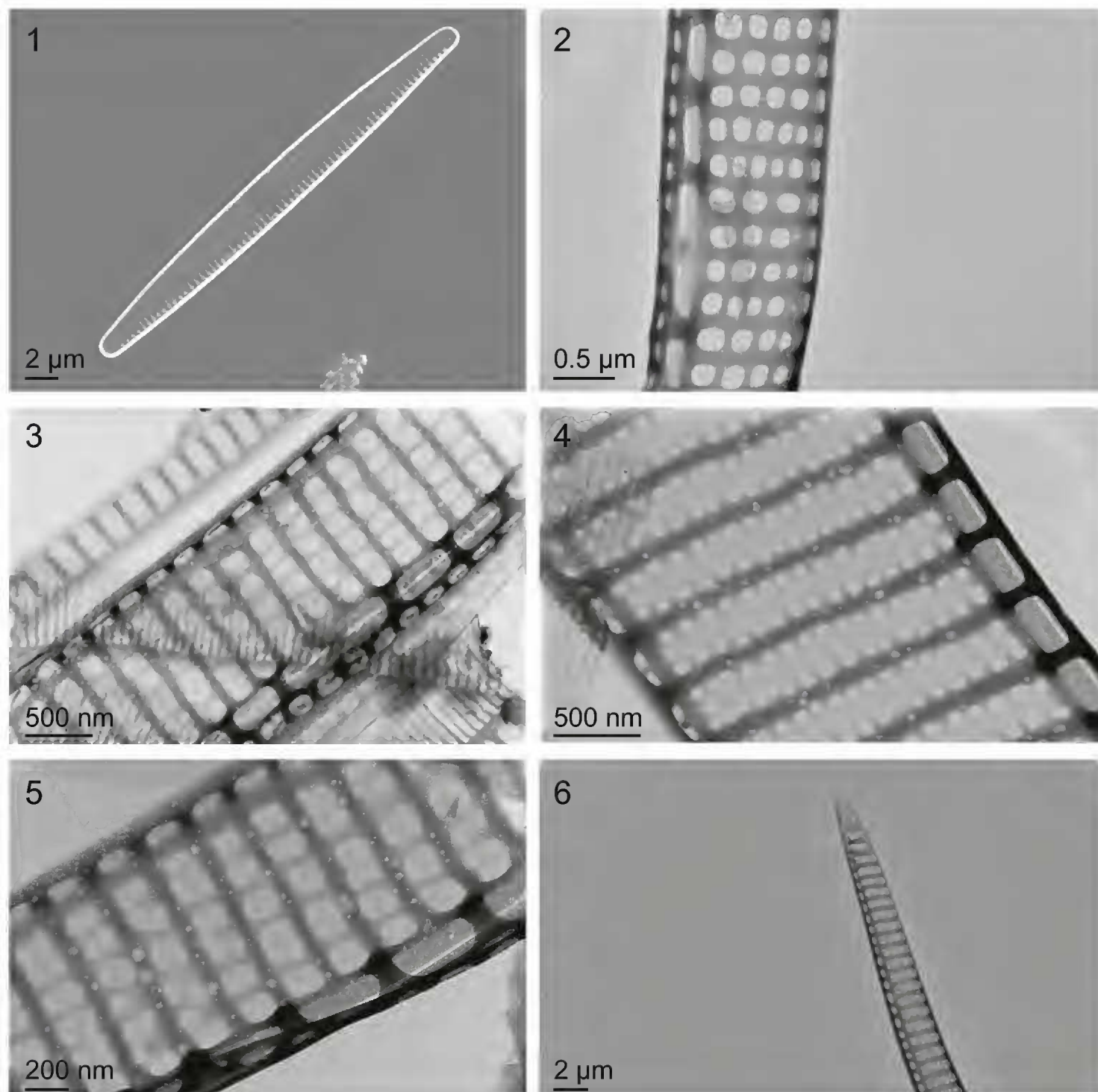


Plate 1. Morphology of *Pseudo-nitzschia* species from the National Park Sistema Arrecifal Veracruzano in electron microscope (1 – SEM, 2-6 – TEM): 1 – *P. brasiliana*, 2 – *P. calliantha*, 3 – *P. cuspidata*, 4 – *P. delicatissima*, 5 – *P. pseudodelicatissima*, 6 – *P. pungens*.

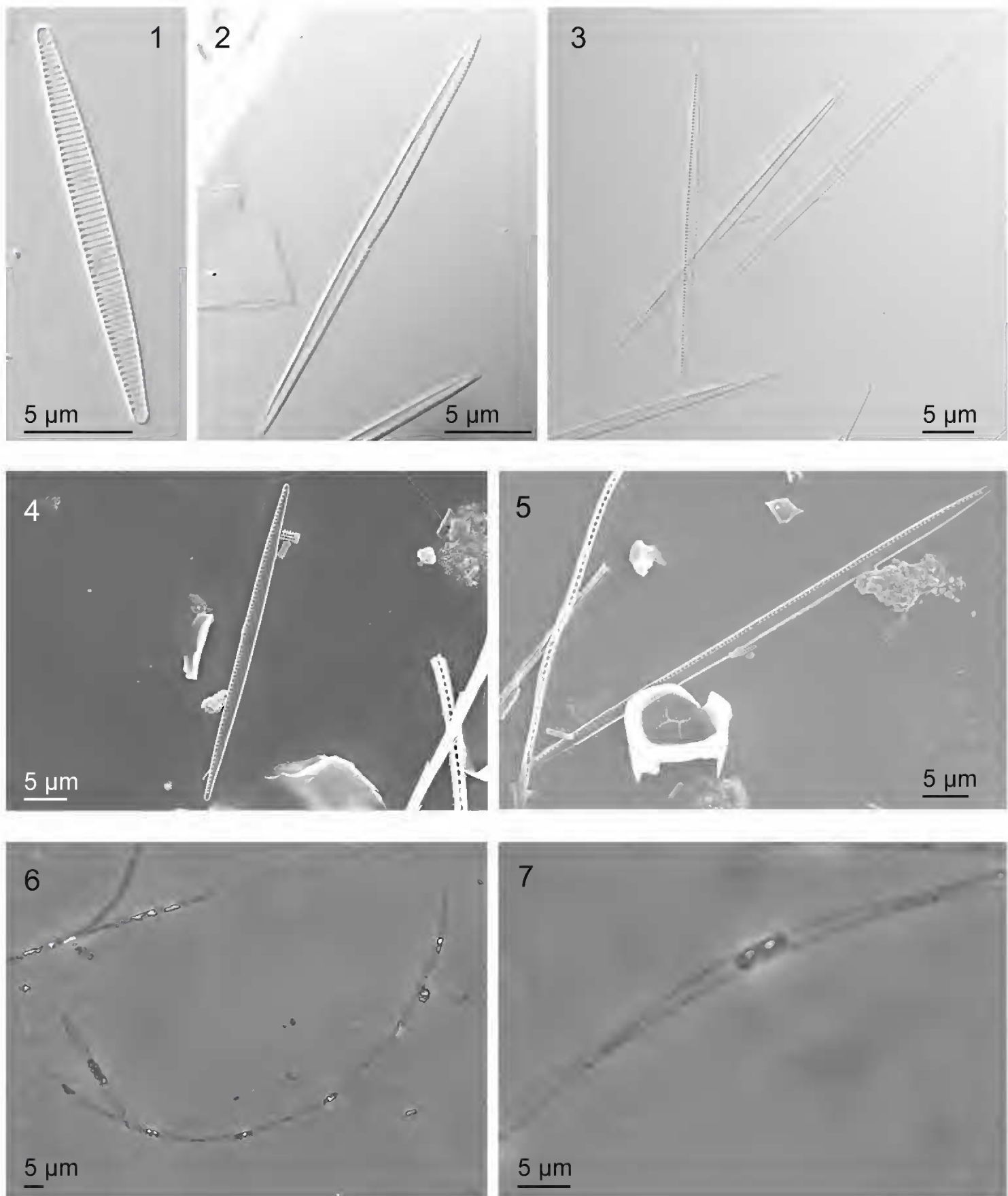


Plate 2. Morphology of *Pseudo-nitzschia* species from the National Park Sistema Arrecifal Veracruzano: 1 (DIC) – *P. brasiliiana*; 2 and 3 (DIC), 4 and 5 (SEM) – *P. “pseudodelicatissima/cuspidata” complex*; 6 and 7 (phase contrast) – *P. cf. subcurvata*.

Taxonomic note. This species belongs to the “*seriata* complex” (Hasle & Syvertsen, 1996). The number of poroids in the specimens from NPSAV is higher than that reported in Quijano-Scheggia et al. (2008) (2.5-3.0 in 1 μm), but is within the range reported by others (3-4 in 1 μm in Hasle, 1965; Takano & Kuroki, 1977; Rivera, 1985). The literature sometimes indicates one or three rows of poroids in 1 μm (Hasle, 1965; Rivera, 1985), whereas only two rows were observed in the cells from NPSAV.

Toxicity. The species is most often characterized as being non-toxic; however, some strains do produce low amounts of DA (Bates & Trainer, 2006). DA was detected in strains isolated from Washington State, USA (Trainer et al., 1998; Baugh et al., 2006) and New Zealand (Rhodes et al., 1998).

7. *Pseudo-nitzschia* cf. *subcurvata* (Hasle) G. A. Fryxell, 1993 (Pl. 2, Fig. 6 and 7)
Bas.: *Nitzschia subcurvata* Hasle, 1964.

Cells are lanceolate, curved in relation to the apical axis: in valve view, convex from one side and concave from another, 28-36 μm long, 1.9-2.5 μm wide, overlapped 1/7-1/8 of the cell length. Striae and fibulae were not distinguished in the light microscope. Cells are solitary or form colonies of 3 to 6 cells.

Taxonomic note. This species belongs to the “*delicatissima* complex”. The cells previously observed by others are 47-113 μm long, 1.5-2.5 μm wide, have 44-49 striae and 12-18 fibulae in 10 μm (Hasle, 1964; Hasle & Syvertsen, 1996).

Biogeographic note. *Pseudo-nitzschia subcurvata* is abundant in the Antarctic (Hasle, 1964; Hasle & Syvertsen, 1996; Scott & Thomas, 2005; Almandoz et al., 2008). The species has been also encountered in the Arctic, Subarctic Pacific and near the eastern Australia (Taylor & Waters, 1982; Crosbie & Furnas, 2001; Flint et al., 2001; Ikävalko, 2003). The species was first reported for the Gulf of Mexico by Aké-Castillo & Okolodkov (2009).

Toxicity. There are no reports about the toxicity of this species.

DISCUSSION

Out of seven species presented in this work, *Pseudo-nitzschia brasiliiana* and *P. calliantha* are new records for the SW Gulf of Mexico. *Pseudo-nitzschia multiseriata*, *P. pungens*, *P. subfraudulenta* and *P. subpacificana* have been previously reported from the Strait of Yucatan (Licea, 1992), and *P. delicatissima* from Veracruz (Santoyo & Signoret, 1988). A similar diversity of *Pseudo-nitzschia* was noted by Parsons et al.

(1998), who reported on the presence of six species of *Pseudo-nitzschia* in the northern Gulf of Mexico: *P. cf. americana* (now known to be *P. brasiliiana*), *P. delicatissima*, *P. pseudodelicatissima* (now known to also include *P. calliantha* and *P. cuspidata*), *P. multiseriis*, *P. pungens* and *P. subfraudulenta*. *P. multistriata* has also been observed in the northern Gulf of Mexico (M. Parsons, pers. obs.). Three of the species found in the northern Gulf of Mexico were not observed in this study: *P. multiseriis*, *P. multistriata* and *P. subfraudulenta*, suggesting that there may be a greater diversity of *Pseudo-nitzschia* present in the NPSAV than captured in this study, or that biogeographic limits of these species may be present. Similarly, *P. subcurvata* was not observed in the northern Gulf of Mexico, possibly due to similar reasons. Wolny & Heil (2005, unpubl. data) reported on the presence of nine species of *Pseudo-nitzschia* in coastal waters of west Florida (*P. calliantha*, *P. cuspidata*, *P. delicatissima*, *P. granii*, *P. heimii*, *P. multiseriis*, *P. pseudodelicatissima*, *P. pungens* and *P. turgidula*), again demonstrating the presence of additional species (*P. granii*, *P. heimii* and *P. turgidula*) and the absence of others (*P. multistriata*, *P. subcurvata* and *P. subfraudulenta*). It is therefore likely that a higher diversity of *Pseudo-nitzschia* is present in the Gulf of Mexico than has been documented to date, and/or that biogeographic limitations may be present for some species in some localities; e.g., *P. multiseriis* was absent in samples >27 °C in coastal Louisiana (M. Parsons, unpubl. data).

The analysis of our samples showed the frequent dominance of *Pseudo-nitzschia* species in net plankton. *Pseudo-nitzschia delicatissima* was one of the three most conspicuous diatoms in the annual cycle of 1975-1976 at Punta Limón, in the central part of coast of the State of Veracruz (Santoyo & Signoret, 1988). *Pseudo-nitzschia* species continued to be a dominant member of the phytoplankton community, dominating the phytoplankton community in NPSAV from May into November 2005, reaching bloom concentrations (1.5×10^6 cells/l) in August 2005 (Okolodkov et al., 2009). This time period was characterized by higher temperature (up to 32 °C) and high precipitation (Okolodkov et al., 2009), suggesting possible nutrient enrichment related to local riverine inputs from the rivers Jamapa (discharging into the NPSAV), La Antigua (to the north-west), and Papaloapan (to the south-east). Just as other studies have demonstrated that *Pseudo-nitzschia* can proliferate in eutrophic conditions (e.g., Dortch et al., 1997; Parsons et al., 2002), continued nutrient enrichment and subsequent eutrophication of the coastal urban zone of Veracruz-Boca del Río-Antón Lizardo from these riverine inputs could result in larger and more frequent *Pseudo-nitzschia* blooms. This study demonstrates that potentially toxic *Pseudo-nitzschia* species are present in NPSAV; thus their increased abundances could lead to an increased risk of ASP

in the study area. The vectors of DA in the NPSAV are still unknown, however, and will require further study. Seasonality of individual *Pseudo-nitzschia* species and their relationships with physical-chemical variables in the context of the entire planktonic phytocoenosis must be one of the future priorities to allow evaluation of the potential risk of ASP in the study area.

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